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OBSERVATIONS ON THE DEVELOPMENT OF *HETERAKIS PAPILLOSA* BLOCH IN THE CHICKEN

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Heterakis papillosa was first studied by Bloch (1782) who named it *Ascaris papillosa.* In 1791 Fröhlich classified it in the genus Heterakis under the name *Heterakis vesicularis.* Raillet in 1885 accepted Fröhlich's generic denomination and maintained the specific name given by Bloch.

Cecal lesions associated with heterakid worms were first described by Blavette (1840) in chickens infested with *H. perspicillum* (Rudolphi, 1803). Létulle and Marotel (1909) described the lesions produced in pheasants by *H. papillosa,* which had already been noted by Galli-Valerio.

The importance of the parasitism of *Heterakis papillosa* was not realized until Graybill and Smith (1920) found that the disease "blackhead" could be produced in young turkeys by feeding them the embryonated eggs of this worm. This discovery has aroused general interest and has led to further investigations concerning both the life history of this worm and the rôle which it plays in the transmission of blackhead. The manifest economical importance of Heterakis in its relation to the latter disease has led me to undertake some experiments in connection with the development and biology of this worm.

Of recent articles which have appeared on this subject, that of Graybill (1921) on the development of *Heterakis papillosa* in the fowl is of especial interest. The result of my experiments, which were well advanced when this article appeared, confirm for the most part the observations of this writer. As certain additional facts concerning the anatomy and development of the worm will serve to contribute to the general knowledge of its life history it was thought advisable to present the results of my observations. The present paper will deal with the anatomy, development, and biology of the worm as well as with the pathological changes with which it is associated.

Description of the Parasite.—*Heterakis papillosa* is a small slender worm of a whitish color, tapering at both ends, especially the posterior. The male measures from 7 to 8 mm., the female from 8 to 10 mm. but not infrequently attains a length of 13 to 15 mm. The living worm of either sex, especially immature forms, are often found tightly coiled in rings or spirals. In this case it is rather difficult to distinguish them in diluted cecal contents. The female often shows a peculiar characteristic curvature, the anterior portion is bent sharply to the dorsal side sometimes making a small loop, while the rest of the body extends in a curve with its convexity to the dorsal side thus giving to the worm the appearance of an elongated interrogation mark. The male may show irregular curvature either to the ventral or to the dorsal side and often shows two or three bends.

The cuticula is smooth and transparent, without transverse striations. Arising from a point near the anterior extremity, two lateral longitudinal ridges are seen which terminate at the posterior extremity in the female in a flattened elongated tail, in the male in a copulatory bursa. These ridges are somewhat of the character of the "wings" of Oxyuris. The anterior extremity is provided with three lips of equal size of which one is dorsal and two ventral. The ventral ones show two small inconspicuous papillae situated at their bases.

The mouth is a simple orifice, followed by a short and narrow oral cavity. On the walls of this cavity and near the oral aperture are three indistinct teeth and at the bottom three other very small teeth are seen, projecting vertically, toward the oral aperture (Figs. 10, 15). The excretory pore is found in the anterior third of the body, slightly posterior to the middle point of the esophagus.

The esophagus, measuring 1 mm., follows the oral cavity described. It is composed of two parts: an anterior portion which is very short and a longer posterior portion. They are separated by a constriction of the walls, showing at this point three small pear-shaped structures with their narrow extremity pointing toward the lumen of the esophagus. The anterior portion of the esophagus, as well as part of the oral cavity, is surrounded by a thickening of the muscular tube, thus forming a definite pharyngeal bulb. The posterior portion possesses a larger dilatation at its posterior extremity. This dilatation or esophageal bulb shows a chitinous structure, which in early stages of development, and in macerated larvae, is found to be composed of two cup-shaped pieces with their expanded portions opposed to one another, and separated by a third flattened intermediate piece (Fig. 9). In the adult this esophageal bulb has the shape of a top with its dilated portion toward the posterior extremity and includes, in addition to the chitinous pieces, some cells which appear to be of a glandular nature. From the anterior extremity of the chitinous bulb, three slender chitinous strip-like processes extend anteriorly along the inner aspect of the muscular esophagus, to the pharyngeal bulb. All these structures are better seen when the worm has been macerated in water for some time.

The intestine extends posteriorly from the pharyngeal bulb as a simple tube with a dilatation at the anterior extremity, which sometimes is visible to the naked eye as a transparent spot. The gut, which in both sexes extends along the dorsal side of the worm, becomes narrow posteriorly and bends ventrally to terminate in the female in the anal opening which is situated about 1 mm. from the posterior extremity. In the male the gut joins the genital tract to form a cloaca, which opens 0.26 mm. from the tip of the tail.

The female genital apparatus is formed by a double system of ovaries, oviducts, and uteri, which fill most of the remaining space between the intestine and the body wall. The ovaries are followed by the oviducts, which at their junction with the uteri show an elongated receptaculum seminis. The vagina consists of a muscular tube, of which the portion immediately following the junction of uteri serves as an ovijector. The vagina opens at the genital pore a little posterior to the middle portion of the body. The arrangement of the terminal portion of the female reproductive system is shown in figure 22.

In the male the copulatory bursa is formed by the posterior expansion of the lateral wings, supported by papilliform rays, and is followed by a short tail. Twelve papillae are distributed on each side of the caudal extremity as follows: three around the sucker, one anterior, one lateral and a third near the anus; five adanal, two toward the posterior extremity, and the remaining two lying between these groups (Fig. 14). The sucker, which measures about 60μ in diameter, is situated anteriorly to the anal opening and is provided with a circular chitinous rim exhibiting a papilliform nodule in its posterior border. At its base it possesses a glandular structure (Fig. 27) which secretes a sticky substance.

The spicules are unequal in size, the shorter one measuring about 0.65 mm. in length or approximately one-third that of the longer one which is 2 mm. long. They are enclosed in separate sheaths which arise one on each side of the intestine but posteriorly pass to the dorsal side and, following the curvature of the terminal portion of the gut, bend ventrally to coalesce at the outlet of the cloaca. The shorter spicule is tubular and possesses two lateral flanges (Figs. 13, 13a). The tip has a characteristic twist and is often protruded outside of the body (Fig. 12). The longer spicule is also tubular but possesses only one lateral flange (Figs. 13a, 25). The tip is sharp and straight and is often hidden inside of the body.

The male reproductive system shows a single tubular testis followed by the spermatic duct which terminates in an elongated dilatation or seminal vesicle. The seminal vesicle is followed by a muscular duct which extends along the ventral side of the last portion of the intestine and, like the latter, opens into the cloaca (Figs. 27). In its last portion it possesses some ventrally situated cells which seem to be of a glandular character.

The freshly laid egg is symmetrical, smooth, ovoid, and unsegmented. According to measurements it is 68 to 75μ in length by 36 to 38μ in width. It possesses a thick shell, showing a thickening at one of its extremities in which a lenticular clear space is sometimes seen. The contents of the egg appears as a dull yellowish granular material, which together with the enclosed nucleus fills the egg completely.

Its development outside the host is greatly influenced by environment and it is apparently very resistant to unfavorable influences. In the experiments I used worms from different lots of chickens and turkeys. The worms were washed in several changes of sterile salt solution, and chopped into small bits, which were kept moist at room temperature in Petri dishes. Under these conditions the ova were embryonated in from 9 to 12 days. Some lots, kept in wide-mouthed test tubes under a rather high column of fluid, were found to take about three weeks to mature and at this time a large percentage of the eggs were usually undeveloped and others were disintegrating. A small amount of moisture appears to be more favorable for development than a large volume of fluid. Heavy growths of bacteria and fungi also seem to interfere with development, retarding it and causing many of the eggs to disintegrate. The large number of amoebae which have appeared in practically every culture does not seem to have any effect on their growth.

Unsegmented eggs failed to develop when placed in the ice-box for several days but proceeded to do so when transferred to room temperature. Fully mature eggs were exposed out of doors in a Petri dish to the alternate freezing and thawing of winter weather from Dec. 9, 1921, to Jan. 20, 1922, when some showed larvae still alive.

Embryonated eggs were placed in the hollow of hanging drop slides, allowed to dry at room temperature, and were subsequently examined at intervals of 24 hours after adding a small quantity of salt solution. It was found that after the ninth day the larvae, when freed from the egg shell, were motionless and easily broken. Some showed a folding of the cuticula. By the twelfth day all of them were motionless, granular, flattened, and apparently dead.

The resistance of non-embryonated eggs to various antiseptic solutions of different concentrations was tested according to the procedure of Yoshida (1920). In this experiment the following chemicals were used in the concentrations indicated: Hydrochloric acid, 10 and 15 per cent.; sulphuric acid, 10 and 12 per cent.; nitric acid, 1 and 1.5 per cent.; corrosive sublimate, 1 and 1.5 per cent.; formalin, 10 and 15 per cent.; acetic acid, 10 and 12 per cent.; phenol, 0.5 and 0.6 per cent. In phenol and hydrochloric, sulphuric, and acetic acid the eggs did not develop at all, some were promptly destroyed, others showed vacuolization of their contents after two weeks of exposure. Eggs put into corrosive sublimate were soon destroyed, while some of those in formalin began to develop, but a large percentage, however, soon showed degenerative changes and all failed to attain complete development. In 1.5 per cent. nitric acid many eggs developed after three weeks and were kept alive in this solution for four months (March 9 to September 23). These eggs were eventually all used in feeding experiments, but another lot of eggs have remained alive in 1.5 per cent. nitric acid for seven months (May 19 to Dec. 29, 1921).

Development of the Larva

Different lots of chickens and Heterakis material from various sources were used in the study of the development of this worm in the host. The first lot of chickens when two days old were placed in table-brooder and fed mature Heterakis eggs collected two months earlier. Chicks were then killed at intervals of twenty-four hours during the first days of the experiment and the intervals lengthened thereafter. By carefully examining the cecal contents and scrapings of the mucosa successive stages in the development of the larvae were obtained. The liver and lungs were examined in the fresh state by comminuting the tissue, shaking in salt solution, centrifugating and examining the sediment. After a week from the time of feeding the worm eggs attention was confined to the walls of the cecum and its contents, and both scrapings of the mucosa and paraffin sections of the organ were utilized for study. Sections of other organs were made whenever anything suspicious was detected on gross examination. For the study of the later stages older chickens, kept isolated in order to avoid any additional contamination, were used on different occasions. As a more or less standardized procedure was established in these examinations, the observations will be summarized without detail as to the technique and the conditions of each experiment.

The larva when hatched or freed by cracking the egg shell is colorless. It shows the beginning of the intestinal tract as a granular band extending from the anterior portion, a little posterior to its extremity, to the anus, which is already visible (Fig. 3). According to Graybill, the eggs hatch in the small intestine. They then evidently pass with its contents to the large intestine, from which they enter the ceca, where all the subsequent changes of their development are to be observed. Larvae are found in the cecal contents 24 hours after feeding the eggs. Neither at this time nor subsequently was it possible to demonstrate larvae in other organs. At this stage they measure 180μ, but the morphology of the intestinal tract is, as yet, unchanged. The mouth is clearly visible and shows a subterminal position, as though the dorsal border were a little longer than the ventral. The pharyngeal bulb is undeveloped. The rate of growth in the days that follow is shown in the accompanying diagram (Chart 1).

The small worms now evidently migrate into the cecal glands, for from the second to the fifth day no larvae were found in the cecal contents, although many were present in the scrapings from the mucosa. Up to the fourth day larvae were found in stained sections in the bottom of the crypts of the cecal mucosa (Fig. 29). Subsequent development takes place chiefly outside of the glands in the lumen of the cecum. By the ninth day the rate of growth shows a sudden acceleration (see diagram) and moulting individuals were found. At this stage the mouth is terminal and the posterior extremity shows characteristic differentiation of the male and female. The female exhibits a group of cells in its middle portion, corresponding to the genital organs. At this stage the worms begin to cause slight damage to the epithelium of the cecal glands, into which their anterior third is often found inserted.

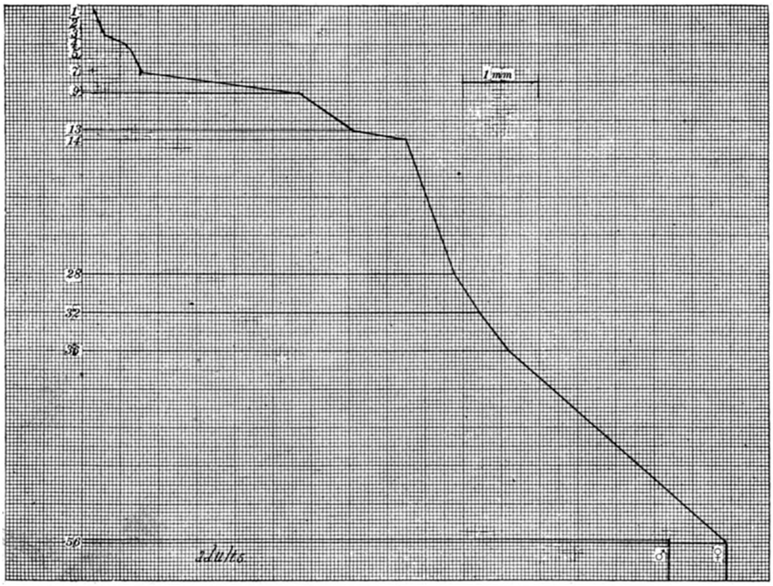

Chart I

On the eleventh day there are several coils of the genital apparatus apparent at the middle of the body. The pharynx shows the beginning formation of the chitinous pieces seen in the adult. At this stage sections of the cecum showed slight but definite lesions, consisting of erosion of the epithelium and minute hemorrhages. Very little or no reaction was found in the adjacent tissues. On the thirteenth day the mouth shows three distinct lips and the pharyngeal bulb has the chitinous apparatus characteristic of the adult, although it is not completely developed (Fig. 9). The female genital pore is readily visible as well as a muscular vagina and several coils of the genital canal (Fig. 11). The excretory pore also is already formed.

One of the chickens used in these experiments contracted blackhead, and when killed thirteen days after the ingestion of Heterakis ova, showed firm, large, hemorrhagic cores, ulcerations of the ceca and typical liver lesions, similar in character to those found in turkeys. Many worms were found in the glandular pits and some embedded in the mucosa (Figs. 30-32).

Later stages of development show nothing of particular interest. As already noted, the worm is subsequently, for the most part, found free in the cecal contents. The genital organs develop slowly. Females with eggs which were found 56 and 61 days after the ingestion of ova are considered to represent the completed adult stage. At this stage the worms begin to lay eggs which are passed in the cecal discharges. Although immature females were found up to the forty-eighth day, these may have been derived from the re-ingestion of eggs that had passed through the alimentary tract without hatching. The data at hand are insufficient to definitely establish the exact period necessary for the completion of development.

Disintegrating adult females were sometimes found in the last portion of the large intestine. The ingestion of fecal material contaminated with one of these dead worms would undoubtedly result in a severe infestation in case sufficient time has elapsed for the eggs to become embryonated.

Habitat and Pathology

Heterakis papillosa is found in the ceca of various birds, and especially of the common fowl. It appears to live free in the intestinal contents, although in some instances adult worms were found lightly attached to the mucosa by their anterior end. When loosened, small inconspicuous holes were left in the mucosa at the points of attachment (Fig. 28); similar depressions were observed in sections of the mucosa. In the course of the examination of the ceca of a large number of chickens and turkeys the worms were occasionally found, in part, included in the cavities of the lymph nodules which in such instances were much enlarged. This may be regarded as an abnormal or accidental relationship since it is not of common occurrence. The reported encystment of Heterakis in the wall of the ceca (Létulle and Marotel, 1909) also represents apparently an unusual relationship of host and parasite.

While the adult forms appear for the most part free in the cecal contents, the younger stages up to the fourth day occur in the glands of the mucosa. Stained sections show also in many instances the head of larger but immature worms buried in the mucosa. The character of the mouth of the adult worm with its peculiarly formed teeth suggests that it may attach itself to the wall of the cecum and accordingly feed upon tissue fluids. A number of worms examined have failed to give the iron reaction. That the worms may, under certain conditions, ingest blood is conclusively shown by one case in which all the worms (about 30) taken from the ceca of a hen, showed a visible red spot at the anterior dilatation of the intestine. Stained sections of these worms showed nucleated red corpuscles in the oral cavity and esophagus. The intestinal contents contained a considerable amount of pigment in addition to masses of bacteria. Unfortunately the fixative used for these worms was not favorable for the demonstration of iron. It should be taken into account, however, that the hen from which these worms were taken was distinctly abnormal. No food had been taken for several days and on postmortem examination a generalized peritonitis was found, probably originating from the oviduct. The ceca were almost empty but showed no lesions.

Even in cases of experimental infestation the pathological changes may consist of no more than a slight flattening of the epithelium in contact with the worm and associated foci of infiltration with eosinophiles. The epithelium may be eroded to some extent with the production of small areas of hemorrhage but the injuries are so minute that they are found only on careful study of stained sections. In a few of the cases sectioned the larvae were found embedded in the tissues but here also the reaction was very slight. In one case showing large numbers of worms either embedded in the mucosa or with the anterior extremity extending into the gland pits, the reaction was masked by a concomitant infection with *Histomonas meleagridis* (Figs. 30-32).

In later experiments nineteen newly hatched chicks were fed with material containing embryonated Heterakis eggs and killed 21 days later.

A large proportion of these chickens developed a typhlitis, the lesions having the general appearance and distribution of the cecal lesions in "blackhead." Nine of these chickens showed thickening of the cecal mucosa, cores and healed ulcerations, but the organism of "blackhead" was not demonstrable in stained sections of any of the lesions.

In experiments carried out by Smith and Graybill (1920) a large percentage of brooder chickens which were fed Heterakis ova developed blackhead, the disease in all cases being limited to the ceca. The blackhead organism was demonstrated in active lesions from the ninth to the fifteenth day after the ingestion of Heterakis ova but older lesions showed no parasites. Since all the chickens in our experiment were killed 21 days after infestation with Heterakis, it is probable that the lesions found represent a late stage of a transient blackhead infection.

SUMMARY

The present study serves to establish certain hitherto unrecognized anatomical features of *Heterakis papillosa.* The observations of Graybill concerning the presence of chitinous teeth about the mouth are confirmed. A muscular thickening of the nature of a pharyngeal bulb is described and the position of the excretory pore is fixed at a point about opposite the middle of the esophagus.

Findings regarding the spicules differ from those of Lauro Travassos (1913) for the same species studied in Brazil. The spicules are described by this author as being equal and only 0.27 mm. long. The spicules in my specimens are not only unequal in size, one being about three times as long as the other, but they are quite different in shape. The shorter one, 650μ, shows two lateral flanges and a characteristic twist of its tip, while the longer one, 2 mm., has only a single flange and shows a uniform curvature.

The results obtained with respect to the resistance of Heterakis ova to drying and to variation of temperature for the most part confirm those of Graybill. The resistance of these eggs to chemicals in solutions of various strengths has also been determined. The fact that they develop uninjured in 1.5 per cent. nitric acid which renders the material external to the shell of the egg bacteriologically sterile, may be of value in determining the source of the protozoon of blackhead.

Heterakis papillosa after hatching in the intestine of the fowl undergoes its further development in the ceca. In no instance were larval worms found in other organs or other portions of the body. Most of the larvae throughout the first stages of their development, that is for the first five days, are to be found buried in the glands of the cecal mucosa. As they increase in size many show the anterior extremity inserted in the mucosa but the mature worms usually are free in the lumen of the cecum. It is apparent from the measurement of a considerable number of worms that the rate of growth is accelerated after each moult. Since females containing eggs were not found until after eight weeks from the time that the eggs were fed, a considerable period of time is apparently required for this species to mature. Not only are the eggs passed in the cecal discharge, but occasionally dead females are also found. After sufficient incubation for the eggs to ripen, such material should produce heavy infestation on ingestion by a suitable host.

Although the adult stage of *Heterakis papillosa* lives in the lumen of the cecum, it is not rare to find these worms with the head buried in the mucosa and probably in rare instances the entire worm may be buried in the cavity of the lymph nodules which occur at intervals in the wall of the cecum. It is probable that the adult worms usually feed upon the cecal contents, but all those derived from a certain case showed blood in the alimentary tract. This may have been the result of unusual circumstances, for the hen from which they were derived had eaten nothing for several days prior to its death and showed a peritonitis at postmortem. The structure of the mouth and the possession of definite teeth suggest the possibility of attachment and the utilization of tissue fluids as food.

No evidence has been obtained that this parasite produces very serious injury to the cecal mucosa of the chicken. As the result of microscopical study it was found that the epithelium in contact with the surface of the worm might be stretched and flattened or in some instances slightly eroded. Small areas of hemorrhage and foci of infiltration with eosinophiles were noted in few instances. In a series of chickens that were not killed until 20 days after the ingestion of Heterakis ova a large proportion showed a marked typhlitis. It is not improbable that this condition represented a late stage of a transient blackhead infection such as that described by Smith and Graybill. At least no protozoa were demonstrable in any of the lesions. A chicken of another series, killed on the thirteenth day, showed blackhead with typical lesions of both liver and cecum. Whether Heterakis produces greater injury in the cecum of the turkey than in that of the chicken is not at present known.

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EXPLANATION OF FIGURES

The camera lucida was employed in all except Figure 28.

EXPLANATION OF PLATE XXVII

Fig. 1.—Unsegmented egg. × 120.

Fig. 2.—Embryonated egg. × 120.

Fig. 3.—Larva free from shell. × 120.

Fig. 4.—Larva in cecum of chicken 24 hours after feeding eggs. ×120.

Fig. 5.—Larva four days old showing subterminal mouth. × 120.

Fig. 6.—Larva five days old. Central spot marking beginning of reproductive system. × 120.

Fig. 7.—Larva six days old. Mouth still subterminal; esophagus clearly differentiated. ×120.

Fig. 8.—Anterior extremity of larva nine days old; mouth terminal. × 120.

Fig. 9.—Anterior extremity of larva 13 days old. × 120.

Fig. 10.—Anterior extremity of adult showing papillae, oral cavity, and teeth. × 220.

Fig. 11.—Genital apparatus of female 12 days old. × 120.

Fig. 12.—Profile of posterior extremity of male showing comparative size of two spicules. ×100.

Fig. 13.—Short spicule with two flanges. ×100.

Fig. 13 a.—Cross section of spicules near cloaca showing long spicule with one flange and short spicule with two included in the same sheath. ×220.

Fig. 14.—Front view of posterior extremity of male showing circular sucker and arrangement of the papillae. × 100.

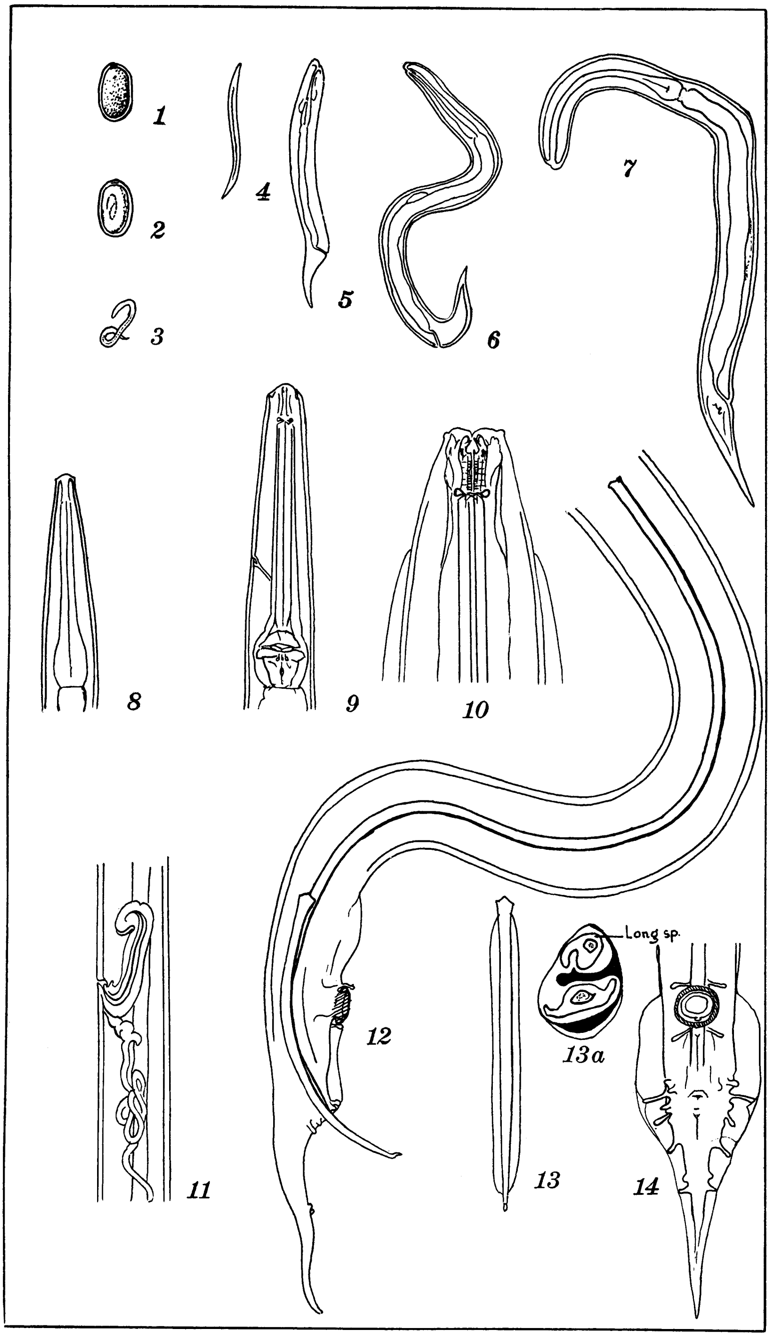


PLATE XXVII

Explanation of Plate XXVIII

Fig. 15.—Stained section of anterior extremity of adult worm showing mouth cavity with teeth, surrounded by pharyngeal bulb. × 60.

Fig. 16.—Longitudinal section of esophageal bulb and of intestinal dilatation. × 60.

Figs. 17, 18 and 19.—Cross sections of esophagus at different levels. × 60.

Fig. 20.—Cross section of female showing intestine, ovary, and uterus. × 60.

Fig. 21.—Cross section of female near genital pore. ×60.

Fig. 22.—Longitudinal section of female showing ovary, uterus, receptaculum seminis, vagina and genital pore. × 60.

Fig. 23. — Cross section of female showing intestine, ovary, receptaculum seminis, and uterus. × 60.

Fig. 24.—Cross section of male showing intestine, testes and long spicule. × 60.

Fig. 25.—Cross section of male showing intestine, both spicules, testes, and vas deferens. × 60.

Fig. 26.—Cross section of spicules at cloaca. × 60.

Fig. 27.—Longitudinal section of male posterior extremity showing vesicula seminalis, intestine, glandular structures and spicule. × 60.

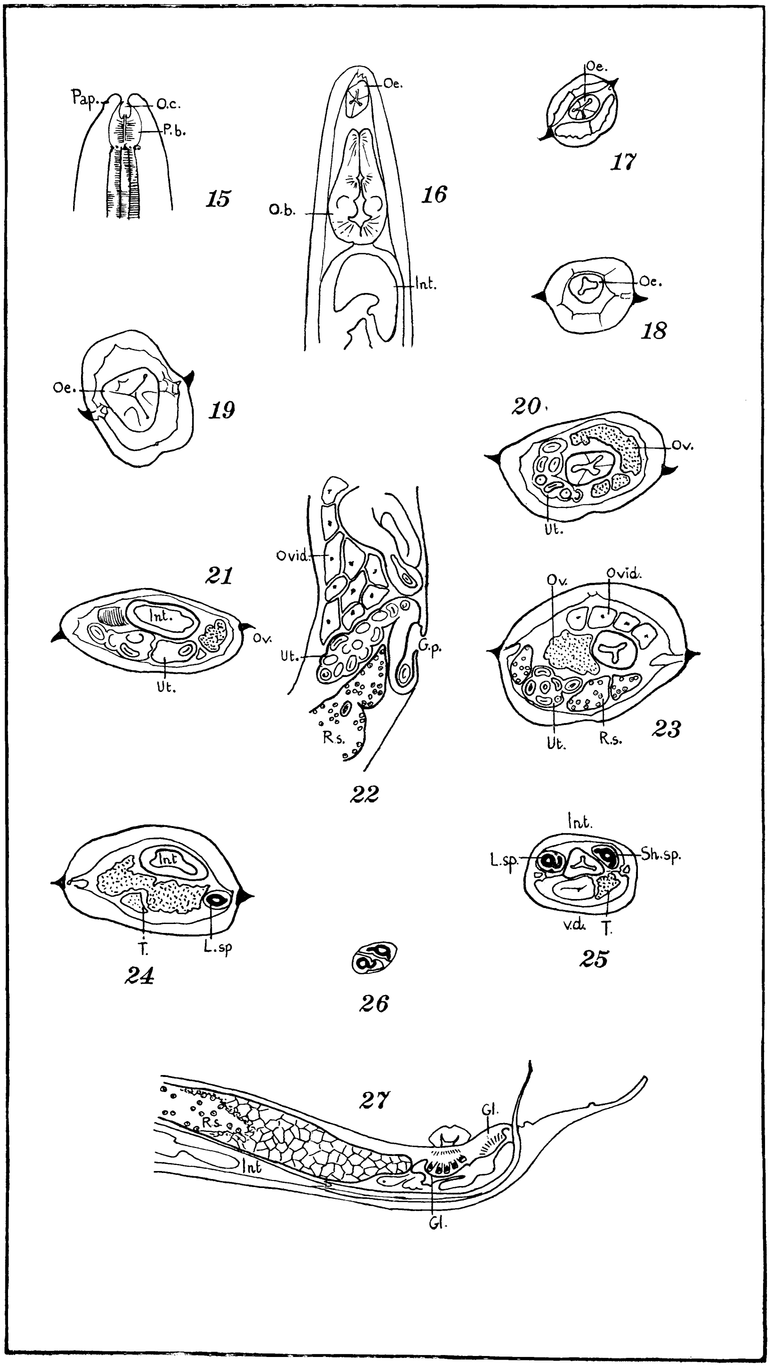


PLATE XXVIII

EXPLANATION OF PLATE XXIX

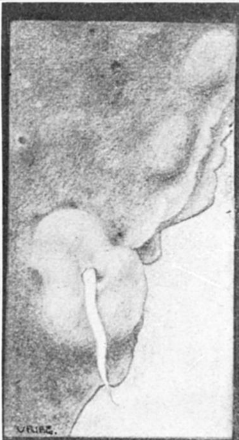
Fig. 28.—Drawing of worm partially included in lymph nodule which shows enlargement. × 3.

Figs. 29-32.—Microphotographs taken at the same magnification. Four day old larvae in gland of cecal mucosa.

Fig. 30.—Section showing larva embedded beneath the mucosa. Blackhead parasites present.

Fig. 31.—Anterior extremity of larva inserted in glandular crypt; numerous blackhead parasites apparent.

Fig 32.—Cross section of larva embedded in tissue and surrounded by blackhead parasites.



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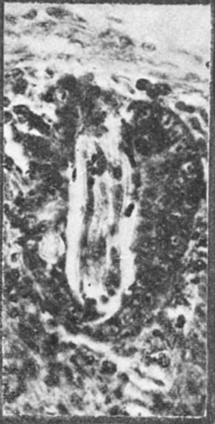

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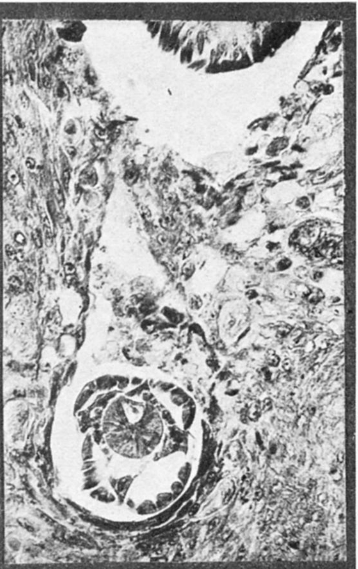

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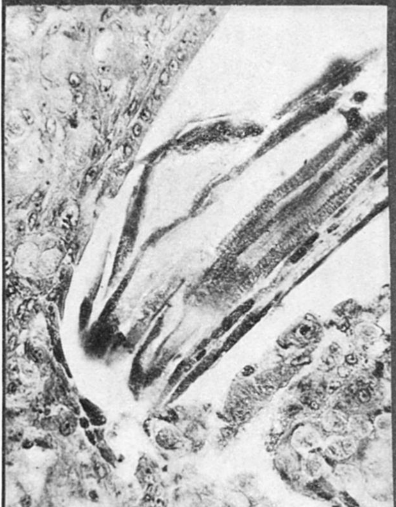

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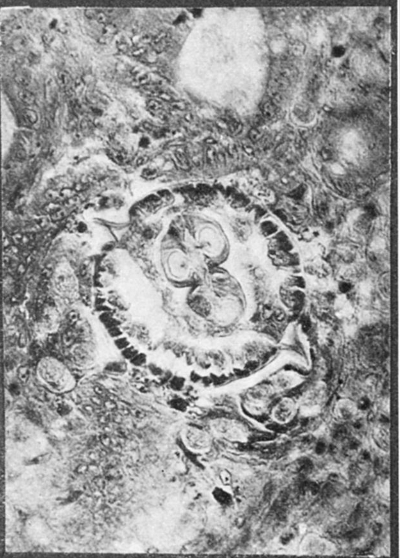

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PLATE XXIX